REMARKS

Claims 1 - 6 and 11 – 24 have been withdrawn. Claims 9 – 10 have been canceled. Claims 7 - 8 has been amended, are pending and are the subject of this Office Action. Support for claims 7 - 8 can be found throughout the specification including the Drawings and claims as filed originally. No new matter has been added.

In order to satisfy the requirement of 35 U.S.C. 119 (b), an English translation of the basic application (Japanese Patent Application, *Tokugart,* No. 2003-166646) is provided with this Amendment.

Applicants respectfully reserve the right to pursue any non-elected, withdrawn, canceled or otherwise unclaimed subject matter in one or more continuation, continuation-in-part, or divisional applications.

It is submitted that the claims, herewith and as originally presented were in full compliance with the requirements of 35 U.S.C. § 112. The amendment of the claims, as presented herein, is not made for purposes of patentability within the meaning of 35 U.S.C. §§ 101, 102, 103 or 112. Rather, this amendment is made simply for clarification and to round out the scope of protection to which Applicants are entitled. Furthermore, it is explicitly stated that the herewith amendment should not give rise to any estoppel.

Reconsideration and withdrawal of the rejections of this application in view of the amendments and remarks herewith, is respectfully requested, as the application is in condition for allowance.

Applicant now turns to comments made by the Examiner in this Office Action as follows.

Office Action

1. The Examiner states, "Claims 8-10 are objected to because of the following informalities: Claim 10 has a typo "claims 7" instead of "claim 7".

Claims 8-10 have "Claim" on line 1 that should begin with small case "c".

Regarding claim 9, acronyms "FGF2", "FGF9" and "CNTF" recited should be spelled out for clarity.

Appropriate correction is required.

Applicants have amended claim 8 to rectify the recitation of the word "Claim" to read with a lower case "c" in the word "Claim", thereby obviating the Examiner's objection. Claims 9 and 10 have been canceled also obviating the Examiner's objection.

2. The Examiner states, "Claims 7-10 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method for producing retinal nerve cells comprising isolation of iris pigmented epithelial cells (IPE) and performing adherent culturing of the IPE with DMEM/F12 or EMEM, comprising FGF2, FGF9 or CNTF for differentiation of IPE to retinal nerve cells, does not reasonably provide enablement for a method for inducing differentiation of IPE to retinal nerve cells with any serum-free culture medium. The specification is not enabled as broadly claimed, because the culture medium and other essential factors required for inducing directed differentiation of IPE to retinal cells is not recited in the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are drawn to a method for producing retinal nerve cells by isolating and differentiating iris pigmented epithelial cells derived from a bird or a mammal, wherein the differentiation is induced by adherent culturing in a serum-free culture medium containing one of FGF2, FGF9 and CNTF at a concentration of 1-100 ng/ml (claims 7-9). The claims also recite that the density of iris pigmented epithelial cells in the medium at the start of the adherent culture is 1×10^5 cells/cm^2 or less (claim 10).

The factors to be considered in determining whether a disclosure would require undue experimentation include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or

unpredictability of the art and, (8) the breadth of the claims. In re Wands, 8 USPQ2d, 1400 (CAFC 1988).

The specification of the instant application teaches that the iris pigmented epithelial (IPE) cells, similar to retinal pigmented epithelial cells and ciliary epithelial cells, are derived from the neural plates (page 4, para 2). The specification also teaches that the IPE cells can be isolated from an eyeball and further subjected to adherent culturing in a serum-free culture medium for inducing differentiation to retinal nerve cells (page 9, para 2), wherein the adherent culturing corresponds to a monolayer culturing step as shown in step S13 of Figure 3. Additionally, the specification states that step S13 can be performed using any "publicly known conventional culture medium" "which makes it possible to induce differentiation of iris-pigmented-epithelial-cell-derived neural stem/progenitor cells into neural cells", for example DMEM/F12, DMEM, EMEM, etc. (page 29, para 2-3). Example 1 of the instant specification demonstrates the induction of differentiation of IPE cells into retinal nerve cells by culturing in serum free DMEM/F12 culture medium with N2 supplement and growth factor FGF2 at 20ng/ml (page 39, para 5). However, the specification does not teach any methods or working examples to indicate that all possible serum-free culture medium can be used for the induction of differentiation of IPE to retinal nerve cells. Undue experimentation would be required of a skilled artisan to determine the specific medium that will have the required constituents in the required concentration for obtaining the claimed directed differentiation of IPE cells to retinal nerve cells.

It is well-known in the art that endogenous and exogenous factors govern the expansion, maintenance and differentiation of stem cells in vitro, a prime one being the cultivation condition. For example, Mokry et al teach that in case of adherent cultures of neural stem cells, various factors affect cell differentiation and the ratio of the resulting cell types. Modifications in culture conditions influencing cell differentiation include medium constituents like serum, growth factors, hormones, differentiation factors, etc. Mokry et al provide a cautionary note stating that "a change in cultivation condition resulted in cell death that reduced the numbers of cells that differentiated" (Acta Med 50: 35-41, 2007; page 39, para 2). However, the relevant literature, does not teach that iris pigment epithelial cells can be induced to differentiate into retinal nerve cells by culturing the IPE cells with any serum-free culture medium as broadly claimed. The skilled artisan will not be able to make and use the claimed invention, thereby entail undue experimentation.

Please note that this rejection can be overcome by reciting the specific medium and other essential constituents required for performing the claimed method of inducing differentiaion of IPE to retinal nerve cells in the independent claim.

Due to the large quantity of experimentation necessary for inducing differentiation of IPE cells to retinal nerve cells using any serum-free culture medium and any factor at any concentration; the lack of direction/guidance presented in the specification; the complex nature of the invention; the unpredictability of reproducible differentiation of stem cells precipitated by changes in the cultivation conditions or medium constituents; undue experimentation would be required of the skilled artisan to make and/or use the claimed invention.".

In addition, the Examiner states, "Claims 7-10 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are drawn to a method for producing retinal nerve cells by isolating and differentiating iris pigmented epithelial cells derived from a bird or a mammal, wherein the differentiation is induced by adherent culturing in a serum-free culture medium containing one of FGF2, FGF9 and CNTF at a concentration of 1-100 ng/ml (claims 7-9). The claims also recite that the density of iris pigmented epithelial cells in the medium at the start of the adherent culture is 1×10^5 cells/cm^2 or less (claim 10).

The specification of the instant application teaches that the iris pigment epithelial (IPE) cells, similar to retinal pigmented epithelial cells and ciliary epithelial cells, are derived from the neural plates (page 4, para 2). The specification also teaches that the IPE cells can be isolated from an eyeball and subject to adherent culturing in a serum-free culture medium for inducing differentiation to retinal nerve cells (page 9, para 2), wherein the adherent culturing corresponds to a monolayer culturing step as shown in step S13 of Figure 3. Additionally, the specification states that step S13 can be performed using any "publicly known conventional culture medium" "which makes it possible to induce differentiation of iris-pigmented-epithelial-cell-derived neural stem/progenitor cells into neural cells", wherein the culture medium can be for example DMEM/F12, DMEM, EMEM, etc. (page 29, para 2-3). Example 1 of the instant specification demonstrates the induction of differentiation of IPE

cells into retinal nerve cells by culturing in serum free DMEM/F12 culture medium (page 39, para 5). However, the brief description in the specification of three examples of serum-free culture medium (DMEM/F12, DMEM, EMEM), does not provide adequate written description of an entire genus of serum-free culture medium that would be able to induce differentiation of IPE cells by adherent culture as broadly claimed. To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of specific physiological characteristics, physical and/or chemical properties, functional features, structure/function correlation, or any combination thereof. However, in this case, the specification has not shown a relationship between the claimed genus of serum-free culture medium.

Vas-Cath Inc. v. Mahurkar, 19USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed" (See page 1117). The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed" (See Vas-Cath at page 1116).

The skilled artisan cannot envision the entire genus of serum-free culture media, of the encompassed methods, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method. Adequate written description requires more than a mere statement that it is part of the invention. See Fiers v. Revel, 25 USPQ2d 1601 at 1606 (CAFC 1993) and Amgen Inc. v. Chugai Pharmaceutical Co. Ltd., 18 USPQ2d 1016.

One cannot describe what one has not conceived. See Fiddes v. Baird, 30 USPQ2d 1481 at 1483. In Fiddes, claims directed to mammalian FGF's were found to be unpatentable due to lack of written description for that broad class.

Therefore, only methods of adherent culture using DMEM/F12, DMEM or EMEM, but not the full breadth of the claim meets the written description provision of 35 U.S.C. §112, first paragraph. Applicant is reminded that Vas-Cath makes clear that the written description provision of 35 U.S.C. §112 is severable from its enablement provision (see page 1115).

Applicants have significantly amended claim 7 to include specific limitations that would not require undue experimentation and for which sufficient written description is provided in the specification. Claim 8 which is dependent from claim 7, also includes include the same specific limitations that would not require undue experimentation and for which sufficient written description is provided in the specification. Claims 9 and 10 have been canceled. Applicants respectfully request reconsideration.

> 3. The Examiner states, "Claims 7-10, are rejected under 35 U.S.C. 103(a) as being unpatentable over Kosaka et al. (Exp Cell Res 245: 245-251, 1998) in view of Haruta et al., (Nat Neurosc 4: 1163-1164, 2001).
>
> The claims are drawn to a method for producing retinal nerve cells by isolating and differentiating iris pigmented epithelial cells derived from a bird or a mammal, wherein the differentiation is induced by adherent culturing in a serum-free culture medium containing one of FGF2, FGF9 and CNTF at a concentration of 1-100 ng/ml (claims 7-9). The claims also recite that the density of iris pigmented epithelial cells in the medium at the start of the adherent culture is 1 x 10^5 cells/cm^2 or less (claim 10).
>
> Kosaka et al. teach the isolation of iris pigmented epithelial cells (IPE) from chicken eyeballs in Eagle's MEM (page 246, column 1, "Preparation of cell"). Kosaka et al. further teach that the IPE cells are depigmented and seeded for transdifferentiation to lens tissue cells at a cellular density of 0.5-1 x 10^4 cells/cm^2 using monolayer cell culture (page 246, col 1, para 1-3). The reference also teaches that basic fibroblast growth factor (bFGF) or FGF2 in the culture medium at a concentration of 1-30 ng/ml, promote growth and differentiation of IPE cells (page 248, Figure 4).
>
> Kosaka et al. do not teach differentiation of IPE to retinal nerve cells in a serum free culture medium.
>
> Haruta et al. teach the plating and maintenance of iris tissue from adult rats in serum free culture medium containing bFGF or FGF2, resulting in the proliferation of cells as a monolayer (Figure 1a, page 1163, para 2). Haruta et al. also teach that the iris derived cells are positive for a retinal ganglion cell marker, neurofilament 200.
>
> It would have been, therefore, obvious to the person of ordinary skill in the art at the time the claimed invention was made to modify the method of inducing differentiation of IPE cells to lens cells by adherent or monolayer culture method in medium

containing serum as taught by Kosaka et al., to the monolayer culture in a serum free medium of Haruta et al., whereby the iris derived cells differentiate to retinal cells expressing neuronal antigen (i.e. inherently retinal nerve cells). The person of ordinary skill in the art would have been motivated because IPE and the neural retina have a common developmental origin, thereby giving rise to retinal neurons (Haruta et al. page 2163). Also, neurofilament being a retinal ganglion cell marker, its expression indicates retinal cell differentiation. Furthermore, a person of ordinary skill in the art would be motivated to use serum-free culture medium because serum is known to contain a mixture of various constituents including different growth factors that would induce differentiation to a non-specific mixture of cells, as opposed to a directed differentiation to retinal nerve cells using specific growth factors at specific concentration, as required by the instant claims. The person of ordinary skill in the art would have expected success because the method of adherent cell culture in serum free medium for differentiation of stem cells, was well established and accepted in the art at the time the invention was made.

Thus, the claimed invention as a whole was prima facie obvious over the combined teachings of the prior art.

Applicants have amended claim 7 to read as follows:

A method for producing retinal nerve cells, the method comprising the steps of:

isolating iris pigmented epithelial cells'from an eyeball; and

performing adherent culturing of the iris pigmented epithelial cells with a serum-free culture medium so as to induce differentiation of the iris pigmented epithelial cells into the retinal nerve cells, the iris pigmented. epithelial cells not being subjected to a gene transfer,

the serum-free culture medium when the adherent culturing starts containing at least one of FGF 2, FGF 9, and CNTF with a concentration in a range of 1 to 100 ng/mL,

the iris pigmented epithelial cells in the serum-free culture cmdium when the adherent culturing starts having a cell density of 1×10^5 cells/cm^2 or less,

the serum-free culture medium being a DMEM/FI 2 culture medium, a DMEM culture medium, or an EMEM culture medium.

According to the invention as described above, it is possible to produce retinal nerve cells without conducting a gene transfer.

On the other hand, a technique of the cited reference *Haruta* is related to a method for differentiating the iris-derived cells into th.e neural retina cells (more specifically, retinal photoreceptor cells) by forcibly expressing a Crx gene in the iris-derived cells and culturing these iris-derived cells.

Therefore, the invention recited in the amended claim 7 of the subject application is different from the cited reference *Haruta* in that the present invention uses the iris pigmented epithelial cells not being subjected to a gene transfer.

The cited reference *Haruta* discloses use of the iris-derived cells. However, *Haruta* does not disclose use of the iris pigmented epithelial. cells. Further, according to descriptions in the cited reference Haruta, it is clear that the iris pigmented epithelial cells are not used as the iris-derived cells in the technique of *Haruta*. This is supported by that the iris-derived cells used in *Haruta* do not produce rhodopsin that is a marker of retinal photoreceptor cells unless a Crx gene is transferred and forcibly expressed.. (refer to Figs. 2 and 3 in the cited reference *Haruta)*

On the other hand, according to the present invention recited in the amended claim 7, with the use of the iris pigmented epithelial cells, the iris pigmented epithelial cells are differentiated into the retinal nerve cells without conducting a Crx gene transfer (refer to Examples in Specification of the subject application). This results from the use of the iris pigmented epithelial cells and indicates that the technique of the cited reference *Haruta* does not use the iris pigmented epithelial cells.

The cited reference *Haruta* teaches that the differentiation of the iris-derived cells into the retinal nerve cells (more specifically, retinal photoreceptor cells) can be achieved only when a Crx gene is forcibly expressed in the iris-derived cells. Therefore,

according to the cited reference *Haruta,* a person skilled in. the art understands that it is impossible to differentiate the iris-derived cells into the retinal nerve cells (more specifically, retinal photoreceptor cells) unless Crx gene is forcibly expressed in the iris-derived cells. Accordingly, a person skilled in the art who read *Haruta* would try to forcibly express a Crx gene in the iris pigmented epithelial cells, for differentiating, into the retinal nerve cells, the iris pigmented epithelial cells that is disclosed as the iris-derived cells in the cited reference *Kosaka.*

Therefore, a person skilled in the art could not have anticipated that the iris pigmented epithelial cells not being subjected to a gene transfer can be differentiated into the neural retina cells. Thus, a. person skilled in the art could not have been easily accomplished the invention recited in the amended claim 7 of the subject application that includes the step of inducing differentiation of the iris pigmented epithelial cells not being subjected to the gene transfer into the retinal nerve cells.

In conclusion, we consider that the invention recited in the amended claim 7 of the subject application sufficiently has non-obviousness over *Haruta* in view of *Kosaka.* In addition, we also consider that claim 8 which depends from the amended claim 7 also sufficiently has non-obviousness over *T-Taruta* in view of *Kosaka.*

In view of the above amendment, applicant believes the pending application is in condition for allowance.

CONCLUSION

Applicants submit that all claims are allowable as amended and respectfully request early favorable action by the Examiner. Applicant's representative would like to discuss this case with the Examiner to learn if any outstanding issues remain after consideration of this Amendment. If the Examiner believes that a telephone conversation with Applicants' attorney would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney of record. Although it is not believed that any further fee is needed to consider this submission, the Office is

hereby authorized to charge our deposit account **04-1105** should such fee be deemed necessary.

Dated: March 16, 2009

Respectfully submitted,

By _____

Customer No. 21874

Gregory B. Butler, Ph.D., Esq.
Registration No.: 34,558
EDWARDS ANGELL PALMER & DODGE LLP
P.O. Box 55874
Boston, Massachusetts 02205
(617) 517-5595
Attorneys/Agents For Applicant